A STUDY OF REGENERATION AS EXHIBITED BY MOSSES.

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(WITH PLATES XIX–XX)

I. INTRODUCTION.

THAT the sexual generation of the bryophytes is endowed with a remarkable power of regeneration is a well-known and oft-stated fact. The extent to which this is true for the liverworts has been shown by the investigations of Vöchting[1] "Über die Regeneration der Marchantieen" and of Schostakowitsch[2] "Über die Reproduktion und Regenerationsercheinungen bei den Lebermoosen." As far as the mosses are concerned, the generalizations have been based upon scattered and isolated observations by Schimper, Goebel, and others, and not upon any detailed investigation. The present work has been carried out with the intention of showing to what extent these generalizations in regard to the vegetative reproduction from stem and leaf are true, and also to throw some light on the physiology of regeneration.

Before proceeding with the results of my own investigations, brief mention will be made of some of the observations previously recorded.

II. HISTORICAL.

The first record of the formation of protonemata by the leaves is by Kützing[3] for *Bryum pseudotriquetrum*. The leaves produced an abundant protonema growth and after a period of eight weeks, buds appeared.

Schimper[4] obtained a growth from the basal portion of

[1] Jahrb. f. wiss. Bot. **16** : 367. 1885.

[2] Flora, Ergänzungsband 1894 : 350–384.

[3] Phycologia generalis 282. 1840.

[4] Recherches anatomique et morphologique sur les mousses 19. 1848.

detached leaves of *Funaria hygrometrica*, but no buds were produced. He also makes the very broad statement: "Chaque feuille et même chaque portion de feuille detachée de la plante-mere et placêe dans les conditions convenables peut produire des filaments proembryonnaires, par la multiplication d'une ou de pleuiseurs de ses cellules parenchymatuses." Goebel[5] also mentions the ability of Funaria leaves to produce protonemata, when they are detached and kept moist. Limpricht[6] states that almost every leaf can by proper culture be made to form secondary protonemata. Also in the case of plants with brittle leaves, as *Leucobryum glaucum*, *Barbula fragilis*, *Campylopus fragilis*, and *Barbula ruralis*, one can find in nature on the detached leaves the beginnings of protonemal filaments.

It is to be noted that in all of the cases above mentioned, regeneration only occurred when the leaves were detached from the stem. That this is not necessary in all cases is shown by the observations of Goebel[7] on the leaves of several species. In *Oncophorus glaucus* a thick felt of tangled filaments appears on the fertile summits of the plants, which prevents their further growth and eventually gives rise to patches of young plants. The marginal cells of *Buxbaumia aphylla* leaves are able to produce protonemata which will completely envelop the leaf. According to Limpricht,[8] the apex of the end bud in Leucobryum has been known to produce a protonemal growth, and H. Schulze has observed a luxuriant growth of protonemata from the leaf apices of *Hypnum giganteum*.

Mention should be made here of the formation of brood-bodies on different portions of the leaf, now apex, now costa, in various species of Orthotrichum, Ulota, Barbula, Grimmia, Syrrhopodon, and Calymperes.[9] These brood-bodies are apparently formed in the younger stages of the leaf and are homol-

[5] Sitz.-Ber. d. mat.-phys. Classe d. k. bayr. Akad. d. Wiss. **26**: 463. 1896.

[6] Laubmoose von Deutschland **1**: 64.

[7] Outlines of Classification 173. 1887.

[8] *Loc. cit.*

[9] GOEBEL, Outlines of Classification 172–173. 1887. LIMPRICHT, Laubmoose **1**: 64.

ogous with protonemal productions. They become detached from the leaf and under proper conditions grow out into protonema filaments, although in some cases growth may begin before detachment.

The formation of a protonema and the later production of a new plant has been observed from the calyptra of *Conomitrium Julianum*. According to Goebel [10] the formation was from the inner side, and according to Schimper,[11] from the outer surface. Limpricht [11] has also recorded the production of protonemata by the detached calyptrae of Phascum.

Limpricht [12] ascribes to all parts of the moss plant a very great power of regeneration since he says: "Alle Teile der Mosspflanze besitzen die Fähigkeit, sekundäre Protonema zu erzeugen," and specifically in regard to the stem: "Auch jede Zelle der Stengeloberfläche ist fähig einen Protonemafaden zu bilden." In a great majority of cases, however, an intervention of rhizoid production occurs. The sessile or stalked brood-bodies of *Pleuridium alternifolium* originate from the stem. *Bryum erythrocarpum*[13] produces axillary brood-bodies, and *Webera annotina* and W. *Ludwigii* [14] produce axillary bulbils which detach themselves from the stem and grow without the intervention of any protonemata. Schulze [15] records the production of bulbils by the stem of *Hypnum aduncum* which detach themselves and grow in a similar way. The brood-bodies of Aulacomnium and of *Tetraphis pellucida* also originate from the stem. Mention should also be made here of the work of Müller-Turgau [16] on the production of "Zweigvorkeime."

Not only the gametophyte, but also various parts of the sporophyte are able to produce protonemata. This has been observed by Stahl [17] from the capsules and setae for *Ceratodon purpureus*, and by Pringsheim [18] for *Hypnum serpens*, *H. cupressiforme*,

[10] *Op. cit.* 173.

[11] LIMPRICHT, Laubmoose 1: 65.

[12] *Ibid.*, 61 and 63.

[13] SCHIMPER, Rech. anat. et morph. sur les mousses 19. 1848.

[14] *Ibid.*, 7.

[15] Bot. Centralblatt 31: 382–384. 1887.

[16] Arb. d. Bot. Inst. Würzb. 1: 475–499. 1874.

[17] Bot. Zeitung 34: 690. 1876.

[18] Jahrb. f. wiss. Bot. 11: 1–46.

and *Bryum cæspiticium*, all in artificial cultures, and by Brizi,[19] in nature for *Funaria hygrometrica*. According to Brizi, some of the setae of Funaria which had come into contact with the earth produced an abundant growth of protonemata with numerous buds.

III. METHOD.

In course of the experiments described below three different methods were used. The leaves and stems to be used as cultures were carefully washed in sterilized water in order to render them as free as possible from bacteria and fungi, and then placed either in Petri dishes upon several thicknesses of filter paper which had been saturated with a nutritive solution, or upon pieces of flowerpots placed in crystallizing dishes. In the third method the leaves were placed upon soil in either Petri or crystallizing dishes. The filter paper was carefully sterilized in boiling water and then placed in the Petri dishes which had been previously sterilized in the dry-oven. The pieces of flowerpots were first boiled and then sterilized together with the crystallizing dishes in the dry-oven. The dishes containing the soil were also sterilized in the same way. All of the cultures were supplied with a ¼ pro mille normal nutritive solution, and were kept at a temperature varying between 19–21° C.

IV. EXPERIMENTAL.

In course of my investigations the following species were used: *Mnium rostratum* Schwägr.; *Funaria hygrometrica* Hedw.; *Bryum capillare* Hedw.; *Bryum argenteum* Linn.; *Barbula muralis* Timm.; *Atrichum undulatum* P. Beauv.; *Polytrichum commune* Linn.; *Brachythecium rutabulum* Bry. Eu. and variety; *Leptobryum pyriforme* Schimper; *Phascum cuspidatum* Schreb.; *Ceratodon purpureus* Brid.; *Fissidens bryoides* Hedw.

In addition to these, cultures of *Plagiochila asplenoides* and *Lophocolea bidentata* were made for comparison with those of Schostakowitsch.

[19] Annuar. Istituto Orto botan. Roma **5**: 53–57. 1892.

I. MNIUM ROSTRATUM.

On account of the size of its leaves and the consequent ease of manipulation Mnium presents a very favorable specimen for experimentation. In its power and manner of regeneration it stands alone among all of the species investigated. At first two cultures were made for exposure to light; the leaves were carefully stripped from the stems and in one case placed with the dorsal surface uppermost, in the other with the ventral surface uppermost. These cultures were placed upon a table in the middle of the laboratory. Two similar preparations were made and enclosed in a dark chamber.

After an interval of a week the first appearance of rhizoids from the leaves was noted. An examination of the specimens grown in the light showed that the rhizoids proceeded almost exclusively from the contact surface, and in general from the periphery of the leaf, although they were not entirely absent from the middle and costal region. An examination of the cultures in the dark showed nearly the same manner of growth except that a considerably larger number of rhizoids originated from the side uppermost, the proportion being about one to ten. The rhizoids from the very first, both in light and dark, were devoid of chlorophyll and the cell walls were distinctly brown. As growth proceeded, those in the light developed an abundance of chlorophyll bodies and showed in nearly every case oblique cross walls. In the course of two weeks the rhizoids in the light had branched considerably, while in the cultures in the dark they rarely branched, and the cells were more elongated. At the end of three weeks the first appearance of buds was noted; and in cultures in brighter light in the window after a lapse of two weeks. The buds originated exclusively from the illuminated side and directly from a leaf cell without the intervention of any protonemata. The buds generally made their appearance near the periphery of the leaf, and the cell from which the bud originated had previously given rise to a rhizoid from the contact side. This is shown in cross sections of the leaf in *figs. 2* and *3*. The mother cell of the bud first produces

a protuberance which becomes divided very soon by an oblique wall, and the insertion of the successive walls then follows in rapid order. Buds may occasionally originate as side branches of the rhizoids from either surface, although this is rare in the normal development. At the end of six weeks the specimens in the dark showed no sign of buds, and the long unbranched rhizoids had attained a length of about one centimeter. The peculiar method of regeneration shown in these experiments is especially noteworthy, since Goebel [20] states that the vegetative reproduction of mosses has this peculiarity, that the formation of a new leafy shoot is always preceded by the production of a protonema.

From the above experiments it is demonstrated that there is no inherent tendency to the production of rhizoids or buds from a particular side of the leaf; also that buds are not produced in darkness, either because the photosyntactic processes cannot be active or because light in itself is necessary. The greater production of rhizoids from the free side of the leaf in the dark would indicate that illumination exercised a retarding influence upon their production. The growth of the rhizoids from the contact surface of the leaf may be due either to contact or gravity, or both.

In order to determine the part which contact and gravity play in the direction of rhizoid growth, the following experiments were carried out. Leaves were placed on filter paper and grown in the dark in an inverted position, and in these cultures the same as in the ordinary position, the leaves produced rhizoids mostly from the contact surface. In order to render the supply of moisture of both surfaces as nearly equal as possible, the leaves were grown in a saturated atmosphere. Other leaves grown in both light and dark between two sheets of filter paper showed a production of rhizoids about equally from both surfaces. Again, leaves which were grown in a vertical position produced rhizoids radially in all directions. These experiments then show that the rhizoids are not influenced as to their point of origin by gravity,

[20] Outlines of Classification 170. 1887.

but rather by contact. Leaves were also grown in soil with about the same result except that a greater number of rhizoids originated from the surface of the leaf nearest the air. The formation of buds upon the leaf in the ordinary manner was naturally prevented and when the rhizoids reached the surface of the soil and were exposed to light, they gave rise to an abundance of protonema-like branches and numerous buds.

A culture of leaves with long, sparsely branched rhizoids which had been grown in the dark was removed to the light and allowed to undergo further development. When examined a week later the rhizoids had produced in the apical region an abundance of branches, part of which were still rhizoidal in character. A large number of the branches were, however, distinctly protonemal, the cell-walls colorless, the cross walls perpendicular, the cells short and filled with an abundance of oval chlorophyll bodies. The rhizoids also contained chlorophyll bodies but they were fewer in number and of an elongated lenticular form. An enormous number of buds was also formed, and in one of two ways: either as a direct modification of a side branch from a rhizoid cell, or as a side branch from one of the lateral protonemal branches. This is plainly illustrated in *figs.* *6* and *7*. Occasionally a bud was formed later near the leaf, but the great majority made their appearance towards the distal extremity of the rhizoids.

A question which now presented itself was: Is the continued exposure to light necessary to call forth the production of buds? In order to determine whether buds would be produced by light induction, leaves were grown in bright light for nearly two weeks and then carefully examined to see that no buds had been formed. They were then placed in the dark chamber and after five days the formation of buds was observed. The number was much less than from those leaves in the light, and on account of a lack of food material only a limited growth occurred. Whether this light induction is due to physical or chemical changes in substances already present in the leaf, or to the accumulated products of photosyntax, cannot be stated with certainty, but

the experiment which follows would indicate that the products of photosyntax are not necessary to call forth the production of a leafy shoot.

In order to determine whether the products of photosyntax as obtained from the use of the free CO_2 of the atmosphere are necessary to call forth bud production, a culture of leaves was made in CO_2-free air in an apparatus similar to that figured by Pfeffer.[21] At the end of three weeks the leaves showed a very abundant production of buds. It has long been known that plants are able to use the CO_2 of respiration as material for photosyntax. Since this is so, the above experiment does not prove conclusively that light is necessary to effect physical or chemical changes in material already present, for on account of the size of the Mnium leaf, the CO_2 produced by destructive metabolism would be considerable, and a small amount of carbohydrate food might be formed. Later experiments with other species tend to show that it is the accessible supply of plastic material upon which the production of buds is dependent, and not upon physical or chemical changes in the material already at hand.

Experiments with leaves in colored light by the use of double-walled bell-glasses filled with the solutions of potassium bichromate and ammoniated copper oxide, showed the production of buds as well in the strongly refrangible rays as in the less refrangible. The photosyntax would be greatly suppressed in the leaves exposed to the blue end of the spectrum, and thus this result points to a chemical or physical change in material already at hand. Since Klebs[22] has pointed out a difference in the relation of spore protonemata and leaf protonemata to light in a specific case, we might reasonably expect to find a difference in the leaf productions from different species. Another point which may be noted in the case of the cultures in the rays of different refrangibility is that, in both the strongly refrangible and less refrangible rays, the leaves produced a much greater

[21] Pflanzenphysiologie **1** : 191. 1881.

[22] Biologisches Centralblatt **13** : 646–648. 1893.

number of rhizoids from the surface uppermost. This would tend to corroborate the statement already advanced that light retards the production of rhizoids, since here each culture was only subjected to half the rays of the spectrum.

In all of the cultures the buds only originated from the illuminated side of the leaf, and the question naturally suggests itself: Is this due to illumination or to the negative geotropism of the moss shoot? In order to determine this, a series of leaves was illuminated from below by a mirror, so that light and gravity would be acting in the same direction. After the usual length of time buds made their appearance, and that only from the illuminated surface. Bastit[23] has shown that the moss-plant is distinctly negatively geotropic, but that with illumination from below, the shoots grow towards the light, the influence of gravity being overcome by that of light. This I have been able to substantiate in the case of plants grown from the leaves. Another series of experiments was carried out with leaves illuminated from both surfaces. In order to effect this, the leaves were placed in a Petri dish and irrigated by means of narrow strips of filter paper alternating with rows of the leaves. The dish was placed upon a ring-stand and illuminated from below by a mirror. In this experiment I found that the buds originated from both surfaces, thus showing the dependence upon illumination. In another series of cultures the leaves were placed in a vertical position in the soil and in such a manner that the leaf surfaces were parallel to the incident rays of light. These, as well as the previous experiments showed the production of buds from both surfaces.

In the case of whole leaves the buds appeared only near the periphery and within the leaf margin, the cells of the border never producing any growth. The cutting of the leaves transversely did not alter their power of regeneration, both rhizoids and buds being produced in as great abundance as in the whole leaves. In order to show whether it was possible for the cells from the costal region to give rise to buds, the lateral halves

[23] Rev. gén. de Botanique 3 : 406–411. 1891.

were split away from the costa, and both portions cultivated. The result was that buds appeared from the costal region as well as from the lateral halves, showing that in the whole leaf the power to produce buds was only suppressed. Again with reference to the power of young and old or fully mature leaves to regenerate. Series of leaves from the mature to the very smallest that could be dissected from the end bud were subjected to culture, with the result that the leaves from ordinary size to about half way through the series produced buds and rhizoids in abundance. Those from this point on to the very minute leaves produced only rhizoids, and these mostly from the region of the costa. It was evident that the plastic material was not present in sufficient abundance to produce a further development, or that being an embryonic organ, the young leaf used its available supply of food material towards the growth of its own cells.

So far as I have observed, the leaves of Mnium in nature never give rise to rhizoids when still in connection with the stem. In order to afford experimental proof of this, whole plants were subjected to exactly the same conditions as the detached leaves, but no rhizoid productions resulted. Again, it might be thought that the formation of rhizoids and buds was called forth by the injury to the leaf. That the cutting of the leaf is not effective in the production was shown by those experiments in which the leaves were cut and still left in connection with the stem; even in these leaves no new growth resulted. Another series of experiments was made in which the costa was cut near the base of the leaf while the lateral halves were still left in connection with the stem, with the idea that the severing of the costa might cut off the path for the transport of food material. No rhizoid growth was called forth, and hence the previous experiments show that nothing but the complete separation of the leaves from the stem is able to call forth the power of the leaf to regenerate. When the leaf is still in connection with the stem, the plastic material can be transported to other younger and growing parts; in the detached leaf on the other hand the escape is cut off, and thus may favor the produc-

tion of rhizoids and buds. The simple cutting of the leaf in itself seems to be, however, the important factor, that is, the complete separation of the leaf from the stem affords the stimulus for growth, which is then applied to the production of rhizoids and new leafy shoots.

When the stems of Mnium are stripped of leaves and kept in conditions favorable for growth, they will produce new shoots which originate as axillary branches. As is often noticed in nature, the stems produce an abundance of rhizoids and these in greater abundance from the region of the stem which has given rise to a shoot. In no case, however, was a production of protonemata direct from the stem to be observed, and the rhizoids grew for months without giving rise to any protonemal branches. The production of new shoots from the stems occurred as well in the dark as in the light; in the dark, however, the new shoots produced smaller leaves, and were more slender and elongated. The shoots used for experimentation were laid horizontal, and the lateral shoots grew erect, both in the dark and in the light, thus showing a well marked negative geotropism. The production of the new shoots was not called forth by the defoliation, but only accelerated thereby, since whole plants subjected to the same conditions produced new shoots as lateral branches, according to the manner of branching in nature. The stems also showed quite a distinct tendency to the production of shoots from the region of the morphological apex. Defoliated stems were grown in a vertical position in a moist chamber, part with the morphological apex uppermost, part with it directed downwards. The result was that in the majority of cases the new shoot appeared a short distance below the apical end. In some cases the stems gave rise to several shoots, and some of these were often well removed towards the basal end. The new shoots produced from the stem as well as those produced from the leaves were distinctly positively heliotropic. By reversing the leaf cultures from time to time after they had reached the length of a few millimeters, the stem was made to assume a zig-zag form due to the heliotropic curvatures.

It may be noted here that the leaves generally formed ten to fifteen buds, but only two or three of these continued their development to any considerable size. It has been already noted, that leaves in which the bud production was prevented by darkness, produced protonemata from the apical portion of the rhizoids when subjected to light. In case, however, the normal production of buds direct from the leaf was allowed to be carried out, the rhizoids did not produce any protonemata, and ceased growth soon after the new plants had been formed.

2. FUNARIA HYGROMETRICA.

The production of protonemata by the leaves of Funaria has already been mentioned in the references to the researches of Schimper, Goebel, and Klebs. Goebel states that he obtained protonemata in great abundance from Funaria leaves, but my experiments do not show the leaves to be endowed with a very great power of regeneration. The plants used were taken from the greenhouse and were apparently in vigorous condition. Cultures of leaves were made in the same way as for Mnium, and placed in both light and dark. On an average of about one out of every six leaves showed signs of protonemata. In all the cases noted in the first series of experiments, the growth was entirely from the cells of the base and only from those which had been directly attached to the stem. The cultures which were grown in the dark showed growths of a decided protonemal nature, the cell walls colorless, the cross walls generally a little inclined and cells filled with bodies irregular in outline, and without any green color. The filaments remained long and almost unbranched, and reached a length of about 1^{cm}. Several cells of a filament grown in the dark are shown in *fig.* 9 for comparison with those grown under normal illumination.

In one or two cases the leaves produced structures which were more rhizoidal in nature, and these in the cultures both in the light and in darkness. In all of the cultures no buds were produced in the dark, while under normal illumination they appeared after ten days to two weeks. The protonema

very soon after its origin from the leaf, often gave rise to a bud as a lateral branch, and numerous cases were observed in which this bud formation occurred from the second protonemal cell. This is illustrated in *fig. 8*.

In two cases out of all the experiments which I carried out, I found a protonema production from other than the basal cells, so it would seem that the cells of the basal portion of the leaf are more inclined to produce protonemata than those from other parts. In the preparation of the cultures the leaves were stripped from the stem with a pair of forceps, and occasionally portions of the stem were torn away with them. A very abundant production of protonemata occurred from these portions of the stem. In order to show whether the power of regeneration was localized more in the basal cells of the leaf, a series of cultures was made in which the entire basal portion of the leaves was cut away. These cultures were kept for six weeks, and at the end of that time no formation of protonemata had occurred. That the power of protonema production is not confined entirely to the basal cells is shown by the two cases already mentioned where protonemata were produced from the region of the tip. Hence, the experiments only show that the leaf cells adjacent to the stem produce protonemata more readily.

Whole plants brought under exactly the same conditions as the detached leaves did not produce any protonemata from the leaves, and again plants with the leaves cut away at the tip showed no signs of protonema production. From the experiments it must be concluded that the complete separation of the leaves from the stem is necessary in order to call forth the formation of protonemata.

The experiments with the leaves which had portions of the stem torn away with them showed the stem cells to have a remarkable power of protonema production. A series of cultures was made in which the leaves were entirely stripped from the stems and the stems cultivated in both light and dark. The stems produced new shoots as lateral branches with remarkable rapidity. After a lapse of only three days the new shoots had

reached a length of nearly two millimeters. No distinct tendency to the appearance of the new shoots from the region of the morphological apex of the old shoot could be detected. Generally, however, a shoot was formed just back of the apex, but in the majority of cases they were produced at other points along the stem, and even from the very base. Occurring at the same time with the production of new shoots was an abundant growth of protonemata from the stem for its entire length. The regeneration by new shoots was always in the way of axillary branches, in a manner similar to that which often occurs in nature. The protonemata were not, however, confined to the leaf axils, but grew as well from cells removed from the axillary regions. In the cultures in the light they originated generally from the side of the stem which was uppermost, while rhizoids were produced from the contact side and in greater abundance from the region of the stem which had formed a new shoot. This is shown in *fig. 12*. The cultures in the dark showed very rarely a protonema production, and in neither light nor dark was any bud formation noted from the stem protonemata. In several cases where the receptacles with the perichaetial leaves were placed in culture an abundant protonema production was noted from the end cells of the receptacle. A dissection showed these protonemata to originate from the cells lying between the base of the antheridia, archegonia, and paraphyses, and also from the basal cells of the paraphyses as shown in *figs. 10, 11*. All attempts to obtain protonemata from the paraphyses when separated from the stem were without effect. The material for growth was evidently drawn from the stem, and when this supply was cut off the cells were not capable of independent growth.

In order to determine whether the production of new shoots and protonemata was called forth by defoliation or not, whole plants were placed in exactly the same conditions as the defoliated stems. Regeneration by means of new shoots occurred, but not in the abundance that was noted in the defoliated stems, while no production of protonemata occurred and only occasion-

ally rhizoids. The production of protonemata was then called forth by defoliation; the formation of new shoots was only accelerated by the defoliation.

A fact which must be of importance to Funaria was shown in the experiments in which whole plants and defoliated stems were placed under earth at a depth of 3^{mm}. The stems in both cases formed lateral branches which grew erect from the stems which had been buried in a horizontal position. After a lapse of two weeks these new shoots first made their appearance above the soil. Considering the habitat of Funaria the power of regeneration in this manner is of considerable importance in nature, since the plants often become covered with soil and would otherwise perish.

The new shoots from the stem as grown in dark were about twice as long as in the light cultures, and the leaves were much reduced in size. The cultures in the light showed the new shoots to be strongly positively heliotropic. In the dark the new shoots grew erect from the prostrate stems. Stems were placed in a Petri dish in the ordinary horizontal position, and the dish then inverted. The new shoots curved around so as to grow upwards, showing them to be distinctly negatively geotropic.

3. BRYUM CAPILLARE.

The leaves of this plant show a very remarkable power of regeneration. Cultures of the leaves were made the same as for Mnium, and part placed in the light and part in the dark chamber. At the end of a week the majority of the leaves used had produced new growths, and these mostly from the basal portion of the leaf. The first growth from the leaf cells was of neither a pronounced rhizoidal or protonemal nature; the walls were colorless, the cross walls occasionally perpendicular, but more generally slightly oblique. With exposure to light the filaments tended to a growth of a more decided protonemal nature, the cross walls were predominantly perpendicular in the abundant lateral branches, and quite often in the main axes also, and the cells soon developed an abundant chlorophyll content. With

time the walls of the main axes turned brown, and the chlorophyll content disappeared, so that eventually the main axes, even though exposed to the light, came to resemble rhizoids. With the continued exposure to darkness the filaments soon became brown; no chlorophyll was formed, and the lateral branching was very generally suppressed. In the cultures in the dark no buds were formed, while in the light cultures the first buds were noticed at the end of seven days, with the more abundant production as growth continued. The buds originated as side branches of the main axis soon after the filament had grown from the leaf cell. In the further growth the buds appeared at different points along the main axis and were homologous with the lateral protonemal branches. The lateral branches might also in their turn give rise to buds as lateral branches, and after six weeks an enormous number of new plants were produced in this way.

The protonema production occurred generally from the cells of the leaf base, either from the marginal cells or from those of the lacerated base, more generally than from the cells in the interior of the leaf. Although protonemata originated from the cells removed from the periphery, no distinct tendency to production from a certain side of a leaf was noted. Part of the protonemata would originate from the contact surface and part from the free surface, sometimes more from the contact surface, sometimes more from the opposed surface, so that no constant effect of contact was demonstrated. Leaves which had remained in the dark for two weeks had produced long, sparsely branched rhizoids without any signs of buds. They were then placed in the light, and after the lapse of ten days abundant protonemal branches were produced from the distal portions of the rhizoids, and also an abundance of buds, thus showing that light was necessary for the formation of buds. Luxuriantly growing protonemata without any buds were placed in the dark and allowed to remain for two weeks. The specimens were grown upon pieces of flowerpots, and at the end of the two weeks no buds had been formed, although the protonema from its previous

exposure to light must have contained a considerable supply of plastic material, which was used in continued growth rather than in the formation of leafy shoots. No structures at all resembling rhizoids were produced, and at the end of the experiment the protonemal filaments were beginning to die from lack of food material. From these results it will be seen that in the case of *Bryum capillare* a continued exposure to light is necessary for the production of buds.

In order to determine whether the cells removed from the basal region of the leaf were able to produce protonemata as readily as those of the base, a series of cultures was made in which the leaves were cut transversely through the middle, and both basal and apical portions retained in culture. The basal half of the leaf produced protonemata from both the proximal and distal ends, but only rarely from the cells occupying the interior. The apical half of the leaf also produced protonemata from the cells next the cut base. (*Figs. 17, 18, 19.*) Another series of cultures was made in which the leaves were cut lengthwise, and these showed protonema production from the base and also from the cut margins. These experiments then show that almost any cell of the leaf may grow out into a protonema, but that in the cells with one side next the margin, the tendency to form protonemata is greater than in those cells which are surrounded on all four sides by others.

The experiments with whole plants placed under like conditions as the separated leaves, showed no protonema production whatever from the leaves, and when the tips of the leaves in whole plants were cut away, even then the leaves formed no protonemata. Thus nothing more or less than a complete separation of the leaves from the stem would suffice to call forth the power of the leaf cells to grow out into protonema filaments.

Experiments with leaves grown in blue and red light brought a different result from that found in the case of the Mnium leaves. The leaves in the red light produced buds, apparently with as great readiness as in normal illumination, while in the blue light no buds whatever were formed. When we reflect that

it is only in the red light that photosyntax takes place to any extent, the importance of this process as furnishing material for the formation of buds is at once made evident. That the products of photosyntax are necessary for the formation of buds is shown by the fact that leaves grown in a CO_2-free chamber also produced no buds. The results of these experiments with Bryum leaves accord with those of Schostakowitsch[24] for the foliose Jungermannieæ, and those with Mnium agree partially with the results for thalloid liverworts. Experiments with Marchantia and other thalloid liverworts showed that regeneration occurred in the dark as well as in the light. I have also confirmed these results in the case of Marchantia, but in the case of *Lophocolea bidentata* my results were different from those obtained by Schostakowitsch for the same species. I found that the detached leaves produced buds from the marginal cells of the leaf, and that this production occurs quite abundantly in the dark, as well as when the leaves are exposed to light. This result is more in accordance with the observation of Klebs.[25] According to Klebs the leaves of *Lophocolea bidentata* produced buds in a weak light at an intensity which was not sufficient to produce the germ disk in the case of spore-protonemata. Mention may be made here of the cultures of *Plagiochila asplenioides* leaves. Greenhouse specimens showing every appearance of vigor were used, and the cultures were kept for over two months, but although the leaves remained green and vigorous, no sign of any bud or rhizoid production was observed. This was one of the species which Schostakowitsch grew successfully, and it is apparent from these results that there are conditions of the plant, when although apparently vigorous, the power of regeneration may be suppressed.

The defoliated stems of Bryum produced some protonemata direct from the region of the leaf axil, but in the case of specimens grown in the dark no distinct protonema growths were noted. The abundance of production was much less than in the case of *Funaria hygrometrica*. The paraphyses here also were

[24] Flora, Ergänzungsband 1894: 380–384. [25] Biol. Centralblatt 13: 649. 1893.

able to grow out into rhizo-protonemata by the continued growth of the distal cell. This occurred, however, only when they remained in connection with the stem, all attempts at cultivating the detached paraphyses being to no avail. The stems produced rhizoids quite abundantly, both in light and darkness, and the production was not confined to any particular portion of the stem. From the rhizoids an abundance of buds was formed as lateral branches, and in a light intensity which was not sufficient to produce vigorous protonemata. New shoots were produced by the stems as lateral branches the same as in Funaria. These appeared without any distinct localization of the point of origin, coming now from near the tip and now near the base of the stem. The production of protonemata was due mostly to defoliation of the stem, since only in rare cases was a protonema production noted from the whole plants which were kept in the same conditions as the defoliated stems. Rhizoid production was quite abundant from the whole plants, but the growth in general was more abundant from the defoliated stems. The production of new shoots was not called forth by the defoliation of the stems, but was only accelerated thereby, since whole plants also formed lateral axillary branches, a mode of growth which is often resorted to in nature, the new branches afterwards becoming separated from the parent plant. The whole and defoliated stems, when buried under 3^{mm} of earth and kept moist, also gave rise to lateral branches, which grew in the normal way, and by rapid growth soon appeared above the soil, the same as in Funaria. The importance of this power of regeneration in nature has already been emphasized in the case of Funaria.

The statements in regard to the elongated growth of the new shoots in the dark, with the development of reduced leaves, and the well-marked negative geotropism and positive heliotropism, hold good here as well as for Funaria.

4. BRYUM ARGENTEUM.

The manner of regeneration from the leaves of *B. argenteum* is so similar to that already described for *B. capillare*, that a

detailed account will not be necessary. The whole leaves produced protonemata from the basal portion, and the cut leaves from all of the cut edges. The character of the growth from the leaf cells was practically the same. The formation of buds occurred in abundance in the light cultures, but none in the dark. The formation of protonemata was due to the separation of the leaf from the stem, and not to the mere cutting.

An abundant protonema production occurred from the defoliated stems, the growth taking place from the region of the leaf axil. The protonemal nature was generally suppressed in the dark cultures, only in a few cases long, unbranched, protonema-like growths being noted. The protonemata in the light produced buds in great abundance, and often as lateral branches of the first cell of the protonemal filament. No buds whatever were formed in the dark. The protonema production was called forth by defoliation, since whole plants only produced rhizoids, and not in the abundance which was noted in defoliated stems. As opposed to the other species studied, the defoliated stems did not produce new shoots as lateral branches, while whole plants under exactly the same conditions did. This is presumably explained by the small weak stem, which when robbed of its leaves is not able in itself to afford material for the growth of new shoots, in addition to what is used to produce the abundant growth of rhizoids and protonemata.

5. BARBULA MURALIS.

The leaves of Barbula produce protonemata with great readiness. Cultures of the detached leaves were made for both light and dark, and the best results were obtained from those upon pieces of flowerpot. After a lapse of about a week an abundant growth had appeared in the cultures in the dark as well as in the light. The first growth was colorless, with slightly oblique cross-walls, and no chlorophyll except what was derived from the leaf cell. Those which remained in the light for the entire period soon showed a very vigorous growth, with luxuriant branching and the absence of any bud formation. The walls

of the main axes after a time turned brown and had more of a rhizoid nature. The side branches, although at times slender and tapering and now with oblique cross-walls, now with perpendicular walls, were decidedly protonemal in character and possessed an abundant chlorophyll content. A thick net of interlacing protonemal filaments was obtained from the culture in the light. At the end of ten weeks the network was several centimeters in extent, and notwithstanding the fact that it had been exposed to the light in the laboratory window, no bud formation had resulted. The suppression of bud formation could not have been due to the lack of sufficient light, since as exposed in the window the illumination was quite intense. Up to this time the culture had produced no growths which I could call rhizoids. The cultures which remained in the dark produced only long, very sparsely branched filaments which in their further growth tended more to rhizoidal nature, with no chlorophyll, brown walls, and always oblique cross-walls.

At the end of about eleven weeks the protonemata had given rise to distinct rhizoid branches, and an abundance of buds had been formed. Soon after this the old protonemata began to turn brown and die. During this period of growth, the extensive network of protonemal filaments had not been entirely produced by the direct growth of the originally formed main axes, but a multiplication of the protonemata had occurred. Certain side branches seemed to be specialized for this purpose, since the cells increased in size, developed a very abundant chlorophyll content, rounded themselves somewhat until they were about barrel-shaped, and then separated from the branches either singly or several together. These separated cells then gave rise to new protonemata. Goebel[26] mentions the power of a protonema, species not known, to separate in this way when the culture was allowed to dry. In the case of Barbula, however, the splitting away of the cells was not due to drying out, since the culture was supplied with nutritive solution for the entire period of growth.

[26] Sitz.-Ber. d. mat.-phys. Classe d. k. Bayer. Akad. d. Wiss. **36**: 641. 1896.

The protonemata originated only from the basal cells of the leaf, generally either from the very end cells or from those next the margin. The cells of the basal portion are much longer than those occupying the apical portion, and the question now presented itself as to whether the small cells of the apical half of the leaf were capable of growing out into protonemata. In order to determine this the basal portions were cut away from a series of leaves, and both apical and basal portions retained in culture. The result was that no protonemata were produced from the apical portions of the leaves, while the basal portions only produced protonemata from the cells of the proximal end. The protonemal growth was generally from cells occupying the periphery, but occasionally one originated from a cell a little removed from the margin. These experiments then show the power of regeneration to be confined to the larger cells of the leaf base.

In the material which was accessible to me, most of the stems were bearing young sporophytes and had produced in their normal growth an abundance of rhizoids. The defoliated stems when placed in culture did not give rise to any new shoots and no appreciable production of rhizoids was to be noted.

6. ATRICHUM UNDULATUM.

So far as my knowledge goes, no moss leaves with a structure similar to that of Atrichum leaves have been known to give rise to protonemata.[27] Hence the successful growth of protonemata from these leaves is of the more interest. Four cultures of leaves were made: a series of leaves with the dorsal surface uppermost, and another series with the ventral surface uppermost, both to be placed in the light; two similar series were placed in the dark chamber. At the end of a month the first signs of protonemata were observed and in the course of a week they had grown to a considerable length. An examination of all the cultures showed that the protonemata in every case origi-

[27] Since this was written an article has appeared by Correns (Ber. deutsch. bot. Gesell. **16**: 22–27. 1898) describing the production of protonemata from Polytrichum leaves.

nated from the ventral surface of the leaf without regard to the position which it had occupied in the culture. And further, the examination of the whole leaves showed that the protonemata originated from the cells lying at each side of the lamellæ. The protonema production from this region was quite general throughout the entire length of the leaf.

In order to determine more closely the origin of the protonemata, cross sections of the leaf were made. The sections showed that they originated from the large cells of the costal region lying at the base of the outer lamellæ (*fig. 32*). The first growth from the leaf, although the cross-walls were predominantly oblique, were decidedly protonemal in character and remained so whether the specimens were grown in the light or dark. The branching was often aggregated in a manner altogether unique, as is shown in *fig. 30*, which may be taken as a typical example. In other cases it was more as in ordinary protonemata, but the only difference between the protonemata grown in the dark and those grown in the light, was that in the dark cultures the cells were generally more elongated and devoid of chlorophyll, and the branching less. The cell walls in both cases were colorless.

After about five weeks, buds made their appearance, and always as modifications of lateral protonemal branches. Contrary to what has been described for all of the preceding species the buds were formed in as great abundance in the dark cultures as in the light. In the dark the buds did not attain any considerable size on account of the lack of food material, rarely reaching a length of 1^{mm}. The production of buds in the dark is evidently explained from the nature of the leaf. The lamellæ and the lateral portions of the leaf, since they give rise to no protonemata are able to furnish considerable food material which can be applied to the growth of leafy shoots. These experiments show that in one case, at least, light is not necessary for developing and unfolding the slumbering "Anlage" of which Klebs[28] surmises the existence.

[28] Biolog. Centralblatt **13**:647. 1893.

In order to determine whether a correlation existed between the lateral halves of the leaf and the costal region in the production of protonemata, the lateral halves of a series of leaves were separated from the costal regions, and both retained in culture. Under no conditions were the cells or the lamina able to grow out into protonemata, the cultures being kept several months without any sign of growth. The costal portions after the usual length of time showed a growth of protonemata in the ordinary way, only the number was greatly reduced. The cells of the lamellæ are not able to grow out into protonemata, neither when in connection with the leaf nor when separated. The power of regeneration is thus distinctly localized in the large cells of the costa lying at the base of the outer lamellæ.

Whole plants which were kept under exactly the same conditions as the detached leaves gave rise to no protonemata from the leaves. That the production of protonemata was not called forth by cutting was shown by the experiments in which half of the leaf tip was cut away while the outer half was left in connection with the stem. Under these conditions the portion of the leaf remaining in connection with the stem showed no growth. A complete separation of the leaf from the stem is then necessary to call forth the power of the leaves to produce protonemata and buds.

The defoliated stems of Atrichum when placed under conditions favorable to growth gave rise to new shoots as axillary branches. This regeneration by means of new side branches occurred as readily in darkness as in light. In the dark the shoots grew more rapidly, producing more slender stems with reduced leaves. The tendency to apical production of shoots was not well marked, the shoots appearing at various points along the stem from base to apex. No new production of rhizoids or protonemata was obtained from the stems under any conditions, although the cultures were kept for several months. Whole plants under exactly the same conditions as the defoliated stems produced new shoots as axillary branches but not in as great abundance as the defoliated stems, showing that the production is accelerated by defoliation.

7. POLYTRICHUM COMMUNE.

Cultures of leaves were made the same as for Atrichum, but it was not till the end of about six weeks that the growth of protonemata was observed. The protonemata were similar in nature to those already described for Atrichum, with colorless walls and oblique cross-walls in both light and dark. A peculiar aggregation of branches occurred quite frequently, an example of which is shown in *fig. 36*, thus forming an assimilating organ, while the production of buds came later. In the protonemata grown in the dark the cells were longer, without chlorophyll, and the branching was more or less suppressed.

The protonemata originated exclusively from the ventral surface of the leaf, that is the lamellate side, without reference to the position which the leaves occupied in culture. An examination of the leaves showed that they came apparently from between the lamellæ, but the exact origin could only be determined by means of cross sections. The sections showed that the protonemata originated from the large cells lying just at the base of the lamellæ (*fig. 34*). The protonema production did not seem to be confined to any particular portion of the leaf, but was quite generally distributed over the leaf cells occupying the position above named. The portion of the lamina not covered by lamellæ is small, but the cells from that portion of the leaf as well as the cells of the lamellæ were not able to grow out into protonemata.

The first production of buds was noted at the end of about seven weeks, and, just as in Atrichum, in as great abundance in darkness as in light. The buds originated either as modifications of lateral protonemal branches or in a few cases by the divisions of the end cell of a main protonemal axis. The explanation for the production of buds in darkness, here as in Atrichum, is to be sought presumably in the nature of the leaf, the accessible supply of nutritive material being considerable.

The complete separation of the leaf from the stem was necessary to call forth the protonema formation. The experi-

ments with defoliated stems gave the same results as have already been described for Atrichum.

Mention may be made here of the cultures of *Pogonatum nanum* leaves. Cultures of these leaves were kept for two months without any appearance of protonemata, although the leaves were apparently vigorous.

8. BRACHYTHECIUM RUTABULUM.

The leaves of Brachythecium did not produce protonemata with very great readiness, only about one out of every eight giving rise to protonemata. The first production of protonemata was noted at the end of nearly three weeks. As in the case of Bryum the first growth was neither distinctly protonemal nor rhizoidal. Even in the cultures in the light the main axis soon changed its cell walls to a well-marked brown, while the side branches continued as distinctly protonemal, with generally perpendicular cross-walls, and an abundant chlorophyll content. Buds were formed very soon and consequently the protonemata did not attain any considerable size. In the cultures in the dark the filaments remained long and occasionally branched, with brown cell walls, oblique cross-walls and no chlorophyll. Buds were found after about three weeks, but these were confined to the cultures which had been exposed to the light. The buds originated in all cases as side branches of the main protonemal axes.

The first experiments with the leaves showed that the protonemata originated exclusively from the larger cells lying next the very base. None of the cells removed from the periphery gave rise to a growth, but only those one side of which was next the free lacerated base of the leaf. Leaves were cut transversely, and also longitudinally and all portions placed in conditions favorable for growth. The apical half of the leaf gave rise to no protonemata whatever, while the basal half only produced protonemata from the proximal end, with an origin the same as in the cases of the whole leaves. The portions of the leaves which had been split longitudinally also gave rise to protone-

mata but only from the base and not from the cells lying along the cut margin. The power of regeneration is then located in the basal cells of the leaf the same as in *Barbula muralis*. The production is due to the separation of the leaves from the stem, since whole plants under exactly the same conditions as the detached leaves produced no protonemata, and since when the leaves were cut and still allowed to remain in connection with the stem no growth was called forth.

Essentially the same results were obtained with leaves from a variety of this species, except that the protonemata originated exclusively from the cells of the base which occupied the position next the costa.

The defoliated stems gave rise to new branches as axillary shoots, both from the main axes and from the side branches. These appeared without any apparent regularity with reference to base or apex of the stems, and also as well in the dark as in the light. In the dark the growth was more rapid, producing longer and slenderer shoots with very reduced leaves. Although the stems were cultivated for about two months no protonema production direct from the stems was observed. The production of rhizoids was not general, only here and there a few being produced. Whole plants brought into the same conditions as the defoliated stems, produced new plants in apparently as great abundance. The rhizoid production was about the same, so it was impossible to say that either rhizoid or shoot production was accelerated by defoliation.

9. LEPTOBRYUM PYRIFORME.

The leaves of Leptobryum compare very favorably with those of *Brachythecium rutabulum* in the extent to which they produce protonemata, perhaps not more than one in ten of the leaves used showing the formation of a new growth. The first appearance of protonemata was noted after the leaves had been in culture for nearly three weeks. As in Bryum and Brachythecium, the first growth was semi-protonemal in character. With continued exposure to light and increase in length, it assumed

more and more the protonemal character. Towards the distal end the cells were much shorter, abundantly filled with chlorophyll, and with perpendicular cross-walls. Even in the light the branching remained suppressed and only long, unbranched filaments about 1^{cm} long were produced. In the cultures in the dark the filaments remained distinctly rhizoidal in nature and reached the length of about 1^{cm} after four weeks of growth. When leaves which had remained in the dark for about four weeks were placed in the light, the continued growth of the rhizoids soon became of a more protonemal character, so that a direct transformation of the main rhizoid axes to protonemata was called forth. Even in this case no branching resulted and no buds were produced, either in the light cultures or in the dark.

The protonemata originated from the basal portion of the leaf and generally from some of the cells a little removed from the end. There was no inherent tendency to the production of protonemata from a particular side of the leaf. And, moreover, the growth occurred now from the contact side, now from the free side. In order to determine whether the cells removed from the base had the power of producing protonemata, the leaves were cut transversely and kept in condition favorable to growth for about two months. No growth resulted from the apical portion, and from the basal portion the growth was the same as in the whole leaf. These experiments showed the power of growth to be localized in the cells of the basal half of the leaf. It was also shown that the production of protonemata was only called forth by the complete separation of the leaf from the stem.

Defoliated stems when kept in culture a short time produced an abundance of rhizoids and protonemata from the region of the leaf axils, the protonemata generally being the more abundant. In the dark all of the growths from the stem retained the nature of rhizoids, but when exposed to light the further growth was of a decided protonemal character. The protonema production was not local but occurred in as great abundance from

one portion of the stem as another. Defoliated stems generally produced one or two new plants as lateral branches. The origin of these was not definite, since they appeared now at the base, now at the apex, and at intervening points. Whole plants placed under the same condition as defoliated ones also produced an abundance of protonemata direct from the leaf axil, the same as in defoliated stems. The production of new shoots was also as abundant in the whole plants as in the case of the defoliated stems. Hence, Leptobryum differs from the other species already described in that the protonema production is not called forth by defoliation.

The form of branching of the protonemata of Leptobryum is worthy of note, since it very frequently differs from the ordinary mode. In the normal branching of protonemata each cell is able to form a branch just behind the cross septum. In this case, however, two branches are formed opposite each other and immediately behind the septum (*fig. 48*). The plants which I used for experiments were grown in the greenhouse, and an examination of sterile plants showed that the production of protonemata from the leaf axils was quite general. The side branches of these protonemata often gave rise to strings of conidia-like cells, which broke away from the branch bearing them. The cells generally had assumed an oval form, were abundantly filled with chlorophyll bodies and quite often large oil drops, and possessed besides slightly thicker walls (*figs. 43, 44*). The striking similarity of this growth to conidia formation in fungi will at once be noted from the diagram. Some of these conidia-like cells were placed in conditions favorable for growth and after a lapse of about eight days germination or growth had occurred as shown in *fig. 45*. A great many of the leaf axils, instead of giving rise to protonemata or rhizoids, had produced dark brown, oval, multicellular brood-bodies borne upon a stalk several cells long (*figs. 46, 47*). The rhizoids also gave rise to similar brood-bodies. The conditions for this conidia production cannot be stated. In the artificial cultures when kept moist, this manner of breaking up of the protonemal branches

into single cells did not occur. The plants in the greenhouse were not, however, especially dry.

10. PHASCUM CUSPIDATUM.

The leaves of Phascum produced protonemata with great readiness and in less time than any other species investigated. Cultures were made for both light and dark, part of the leaves in each case being dorsal side and part being ventral side up. At the end of five days both protonemata and buds had been produced in the light, and a careful examination showed that the majority of the growths originated from the ventral side of the leaf in the region of the costa, without reference to the position which the leaves had occupied in the culture. Occasionally a protonema originated from the leaf cells removed from the region of the costa, and now from the contact side and now from the free side of the leaf. Occasionally some of the cells from the region of the base gave rise to distinct rhizoids which showed no tendency to produce protonemal branches, but remained distinctly rhizoidal in nature even in the light. The branching of the protonemata in the light was not very profuse. while in those which remained in the dark all the time, the branching was suppressed to a considerable extent, and the walls very soon turned brown. No buds were formed in the dark cultures, while the cultures in the light had plenty of buds at the end of five days.

When the lateral halves of the leaves were separated from the costa, they also gave rise to numerous protonemata, showing that in the whole leaf the ability of regeneration was present, but that the supply of food material was contributed to the cells of the costal region, which produced protonemata with greater ease. It has already been stated that the majority of protonema production in the whole leaves was from the ventral leaf surface in the region of the costa. Cross sections of the leaves showed that the cells occupying the costal region on the ventral side were thinner walled than the remainder of the leaf cells, hence they produced protonema more readily.

The dorsal side of the costa was made up entirely of thick-walled cells, hence no protonema production from the dorsal side occurred (*fig. 51*).

Whole plants and plants with the tips of the leaves cut away produced no protonemata from the leaves, nothing but a complete separation of the leaves from the stems being sufficient to call forth the formation of protonemata. The protonema production from the costal region occurred throughout the entire extent of the leaf, but that from the apical portion was greater than that from the basal portion.

The defoliated stems produced protonemata directly throughout their entire length, and they were not confined entirely to the leaf axil, any of the surface cells being capable of growth. The defoliated stem generally produced at least one new shoot, and sometimes this originated from the very base of the stem, sometimes from nearer the apex. Whole plants placed in exactly the same conditions produced protonemata directly from the stem, and also new shoots.

II. CERATODON PURPUREUS.

All attempts to obtain protonemata from the leaves of Ceratodon were without effect. The leaves were kept for several months in apparently vigorous condition without any sign of protonema formation. The defoliated stems are however able to give rise to an abundant protonema growth, which originated direct from the region of the leaf axils. Rhizoids were also produced, but they very soon became protonemal in nature in the cultures which are exposed to light. In the dark the growths were of a more rhizoidal nature, and generally remained almost devoid of branches. The protonema production was not local but was general throughout the entire length of the stem. The defoliated stems also produced new shoots as lateral branches. The point of origin was not definite, since they might come at any point between the base and apex of the stem. The production of new shoots occurred as well in darkness as in light. Whole plants placed in the same conditions as the defo-

liated stems, produced new lateral shoots and quite a number of protonemata, hence the protonema and shoot production was not called forth by defoliation.

12. FISSIDENS BRYOIDES.

As in Ceratodon, all attempts to grow protonemata from the leaves of Fissidens were ineffectual. The leaves were kept for three months, and at the end of that time, although in apparent vigor, no protonemata had been produced. The stems, when stripped of leaves, produced rhizoids directly from the region of the leaf axils in both light and darkness. In the light, however, the rhizoids soon grew to possess a distinct protonemal character, but no protonemata originated directly from the stem. The stems grown in the dark produced long, sparsely branched rhizoids, which attained a length of about 1^{cm} after a month of growth. When first examined they possessed only oblique cross-walls, but at this time nearly all showed alternately oblique and perpendicular walls. The oblique walls were the ones first formed and the perpendicular walls were produced later by intercalary division. The great regularity of the alternately oblique and perpendicular cross-walls was due to the fact that each cell had become divided by a perpendicular wall. This fact is mentioned since intercalary division is an exception to the usual mode of protonema and rhizoid growth, and since it affords another example of perpendicular cross-walls being produced in darkness.

No buds were produced from the protonemata grown from the stem, but the stem gave rise to buds, and that in a peculiar way. After one month of culture the stems grown in the light were found to have produced buds directly from the region of the leaf axils, without the intervention of any protonemata. A bud grown in this way is shown in *fig. 53*. A surface cell from the region of the leaf axil produces a protuberance, which instead of growing out into a rhizoid or protonema divides directly to form a bud. This manner of bud formation was observed only in light cultures. Plants with the leaves still

intact also produced buds in the same way, although not in as great abundance as in the defoliated stems. The buds were in the course of time detached from the stem. This manner of bud formation is of interest as affording another example of the production of buds without the intervention of protonemata. It is very probable that buds are produced this way in nature, and the presence of young plants coming from the region of the leaf axil confirms the supposition. The direct growth in nature was not followed however.

Mention may be made here of the attempts to obtain protonemata from *Fontinalis antipyretica*. The leaves and stems were cultivated in a variety of ways: in water, on earth, and with varying amounts of moisture, but no protonemata were obtained from either leaves or stem.

13. GENERAL EXPERIMENTS.

As shown by the foregoing experiments, the production of buds with reference to light and darkness seems to have been in a great measure dependent upon the supply of food material which the leaf could afford. The question which naturally suggests itself at this point is: Can bud production be called forth in the dark by the use of some such carbohydrate food as grape sugar, in the case of leaves which in themselves are unable to produce buds with the absence of illumination? This is a question difficult to solve, because the majority of leaves require a considerable length of time for bud production, and because it is impossible to make perfectly sterile cultures. Repeated attempts were made with various leaves, with every care possible to keep the cultures sterile, but the inroads of bacteria and molds usually destroyed the experiments and thus shut out all chance of success.

In one instance, however, my efforts were successful and that in the case of *Phascum cuspidatum*. The leaves of this moss under ordinary conditions produced protonemata and buds after five days. The rapidity of growth made it favorable for experi-

mentation, and the cultures to which grape sugar was supplied formed buds after two days, both in light and darkness, but in greater abundance in the light cultures. With further growth in the dark, the buds grew to produce shoots, two, three, and in one case five millimeters in length.

Experiments were performed with several of the species which produced protonemata the most readily, to see what effect KNO_3 would have upon the regeneration and manner of growth. *Barbula muralis* and *Phascum cuspidatum* leaves were grown in 1 per cent. KNO_3 without any apparent retardation or change in the manner of growth. *Bryum capillare* leaves produced protonemata, but there was a marked retardation of growth and the filaments did not reach any considerable size. In 2 per cent. KNO_3 *Barbula muralis* still produced a vigorous growth without any marked retardation. The cells were, however, generally shorter, and the branching more aggregated. *Bryum capillare* and *Phascum cuspidatum* produced no growth whatever. In 3 per cent. KNO_3 *Barbula muralis* produced a slight growth, but the filaments did not reach any considerable length.

A series of experiments was also carried out in order to determine the temperature at which protonema formation would occur. For these experiments *Barbula muralis*, *Bryum capillare*, and *Phascum cuspidatum* were used, with the results given in the following table:

	19–21°	24°	27°	29.5°	32°	36°
Barbula muralis	×	×	×	×	×	
Phascum cuspidatum	×	×	×	×	×	
Bryum capillare	×	×	×			

Barbula and Phascum produced protonemata with as great vigor at 32° C. as at 19–21°, the temperature of the ordinary experiments; but at 36° no growth resulted. At 29.5° the Bryum leaves produced no growth but were not killed, since when exposed to the ordinary temperature, protonemata were

produced. At 32°, however, the leaves were killed. At 27° a slight growth resulted but with a very marked retardation. At 24° the growth was to all appearance quite normal.

That moss plants are able to be dried completely for some length of time and still retain their power of regeneration has been demonstrated by Schröder.[29] By way of confirmation *Bryum capillare* was dried thoroughly for three weeks, then moistened and the leaves stripped from the stems and placed in conditions favorable for development. In the same time as usual protonemata made their appearance. *Barbula muralis* was dried for two weeks without the loss of protonema production.

The foregoing experiments have shown that in nearly all conditions, the only requisite for the development of protonemata from rhizoids has been the exposure to light. Either the main rhizoid axis has given rise to side branches which were distinctly protonemal in nature, or the continuation of the main axis has become decidedly protonema-like. There may, however, be conditions in which the rhizoids, even though exposed to light, do not produce protonemal branches. The rhizoids from Mnium leaves, in case the normal development of buds is allowed to be carried out, produce no protonemal branches. In the same way the rhizoids from the stem did not give rise to protonemal branches, but if the growth of the stem is interrupted the rhizoids undertake the regeneration of the plant and produce new leafy shoots and protonemal branches. This manner of growth is quite common when tufts of various plants are inverted so that the rhizoids are exposed to the light and the shoots killed by being covered with soil.

The experiments which I have carried out show that the protonemata do not produce rhizoids with as great readiness as the rhizoids do protonemata. This is in opposition to the view expressed by Frank,[30] since he says in regard to the protonemata: "Eben so leicht kann der Faden wieder in ein Rhizoid sich umwandeln." A protonema of *Bryum capillare* was grown on a

[29] Untersuch. aus d. bot. Inst. zu Tübingen **2**: 15–21. 1886.

[30] Lehrbuch der Botanik **2**: 9. 1893.

piece of flowerpot until a considerable size and vigor was attained, and then placed in the dark. At the end of two weeks no sign of rhizoids was detected. The growth had, however, been considerable from the supply of food material which had been produced in the light.

In another case a luxuriantly growing protonema of the same species as above mentioned was placed upon a piece of flowerpot and one half covered with earth, the other allowed to remain free. Only in one or two cases was a growth of rhizoids noted from the part covered with earth. The same result was obtained with protonemata of *Bryum capillare* and *Barbula muralis* in which one half was covered with a screen of black paper. The protonemata lost their chlorophyll content, but did not develop any distinct rhizoids. From these results it is seen that although exposed to darkness and also grown on earth, a rhizoid production only rarely occurred. A culture of protonema of *Barbula muralis* which was grown in the light produced distinct rhizoids after about eleven weeks of growth. Here then is a case of the production of rhizoids in direct illumination. *Bryum capillare* and *Barbula muralis* leaves were grown under water and a luxuriant protonema growth obtained. It might be thought that growing under these conditions, the protonemata would retain their more algal nature and not produce new leafy shoots, but in the case of Bryum, buds made their appearance after the usual length of culture. There was, however, a difference in the form of growth. In Bryum and Barbula the lateral branches grew quite slender and tapering, while in the cultures on flowerpot they were more robust and of equal diameter throughout. In Barbula these side branches frequently possessed oblique cross-walls, while Bryum generally had perpendicular cross-walls. This manner of growth has been mentioned by Goebel[31] for a protonema of *Physcomitrium pyriforme* when grown in water. He compares these side branches to rhizoids and makes the statement that they evidently correspond to rhizoids. It might be inferred that the lack of rhizoid production in these

[31] Flora **72**: 8. 1889.

cultures was due to the medium of growth, either upon flowerpot pieces or in water. Cultures, which from the beginning were made upon earth, showed essentially the same manner of growth, except that the side branches were robust instead of slender, of equal diameter instead of tapering, and were distinctly positively heliotropic. It was only very rarely that a protonemal branch was found penetrating the soil and becoming rhizoidal. The same result was obtained with protonemata, which were grown either in water or upon flowerpot pieces and then placed upon the soil, the further growth still being without rhizoid development. Luxuriantly growing protonemata from the stem of Funaria were half covered with earth without any appearance of rhizoids. Schimper[32] grew Funaria protonema from the spores which did not show any rhizoid production.

V. SUMMARY.

Considering the various species of moss plants used in the foregoing experiments, there are, notwithstanding the variety of results, many striking similarities in the manner of regeneration, a brief summary of which will be brought together in the following conclusions:

1. The majority of moss leaves used showed a remarkable power of regeneration, producing either rhizoids or protonemata, with the later appearance of new leafy shoots. The rhizoid or protonema production was carried out in both light and darkness.

2. The point of origin of the new growth from the leaf in some cases depended upon contact and illumination, and was independent of gravity (Mnium). In other cases the protonema had a definite origin which was independent of external factors, and depended solely on the leaf structure: from the ventral side of the leaf as in Atrichum, Polytrichum, and Phascum; or from marginal cells and thus independent of contact, gravity, illumination, or position of the leaf.

[32] Rech. anat. et morph. sur les mousses, *plate 1*. 1848.

3. The power of regeneration may be distinctly localized: *a*. In special cells of the leaf base as in Barbula, Brachythecium, and Funaria. *b*. In special cells of the ventral leaf surface as in Atrichum and Polytrichum. In other cases the power of regeneration was quite generally shared by all the leaf cells as in Mnium, Bryum, and Phascum.

4. The structures produced might be all rhizoids in both light and dark as in Mnium and occasionally so in Phascum. They were protonemata in light and rhizoids in the dark as in Bryum, Barbula, Brachythecium, and Phascum, or they were all protonemata in both light and dark as in Atrichum and Polytrichum.

5. Buds were produced under ordinary conditions of cultivation only in light in the following: Mnium, Funaria, Bryum, Barbula, and Brachythecium. In both light and dark by Atrichum and Polytrichum under ordinary conditions, and by Phascum when supplied with grape sugar. The production of buds seemed to be in a measure dependent upon the food supply.

6. Regeneration was called forth in all cases by the separation of the leaf from the stem. Mere cutting of the leaves while in connection with the stem did not call forth the production of protonemata or rhizoids.

7. The majority of moss stems, as well as the leaves, showed regeneration, and that in two ways: *a*. By axillary shoots. *b*. By protonemata directly or by rhizoids, which in the light very soon gave rise to protonemal branches. The stems in two cases had the power of regeneration, while this power was not shared by the leaves (Fissidens and Ceratodon).

8. Production of axillary shoots was not called forth by defoliation of the stem, but was generally accelerated thereby. In some cases the protonema production was called forth by defoliation, in other cases only accelerated.

9. Protonema production was quite general throughout the entire extent of the stem. In some cases the protonemata orig-

inated only from the axillary cells, in other cases from the various surface cells of the internode. The axillary shoots in one case showed a tendency to marked apical origin (Mnium). In the other cases the distribution was quite general.

10. The buds originated from Mnium leaves and Fissidens stems without the intervention of a protonema. When of protonemal origin, they were either modifications of lateral protonemal or rhizoidal branches, or direct modifications of the main axes. The tendency of protonemata to produce rhizoids was not as great as the tendency of rhizoids to produce protonemata.

11. The upper temperature limit for regeneration from the leaves investigated varied from 24 to 32° C. Protonemata were grown in 1 and 2 per cent. solutions of KNO_3. Drying for a considerable length of time did not alter the power of the leaf to produce protonemata.

The investigations here recorded were carried out during the years 1896–7 in the laboratory of the Botanical Institute at Leipzig, under the direction of Herr Geh. Professor Dr. Pfeffer. I wish here to express my thanks to him for aid and many valuable suggestions.

PARSONS COLLEGE,
Fairfield, Ia.

EXPLANATION OF PLATES XIX—XX.

PLATE XIX.

Mnium rostratum.

FIG. 1. Diagram of a leaf to show origin of rhizoids and buds. × 12.

FIG. 2. Cross section of a portion of a leaf showing the origin of a bud from a leaf cell, together with the previously produced rhizoid. × 130.

FIG. 3. Cross section of a portion of a leaf showing a bud at a more advanced stage. × 130.

FIG. 4. Portion of a leaf with rhizoids and a bud produced as a lateral branch of a rhizoid. × 53.

FIG. 5. Rhizoid (*r*) with protonemal branches (*p*) which have been produced after exposure to light. × 130.

FIG. 6. Rhizoid (*r*) with protonemal branches (*p*) showing the origin of a bud from a cell of the main rhizoid axis, and homologous with the lateral protonemal branches. × 220.

FIG. 7. Same as 6, only the bud formation has occurred from one of the protonemal branches direct. × 220.

Funaria hygrometrica.

FIG. 8. Protonema and bud grown from the receptacle. × 130.

FIG. 9. Portion of a protonema as grown from a leaf in the dark. × 130.

FIG. 10. Paraphysis, showing the origin of a protonema from the basal cell. × 53.

FIG. 11. Proximal portion of the same on a larger scale. × 130.

FIG. 12. New shoot, rhizoids, and protonemata growing from a defoliated stem.

Bryum capillare.

FIG. 13. Several cells from the leaf base showing the origin of protonemata, with the formation of a bud. × 130.

FIG. 14. Protonema and bud grown from the leaf. × 130.

FIG. 15. Bud and protonema grown from the leaf. × 53.

FIG. 16. Leaf base showing origin of protonemata and the formation of a new leafy shoot. × 53.

FIG. 17. The basal half of a leaf, showing origin of protonemata from both the proximal and the distal ends. × 53.

FIG. 18. Tip of a leaf showing protonemata growing from the cut edge. × 53.

FIG. 19. Protonemata and leaf cells from the preceding on a larger scale. × 130.

FIG. 20. Protonemata originating directly from the defoliated stem. × 130.

FIG. 21. Paraphysis which has grown out into a protonemal filament.

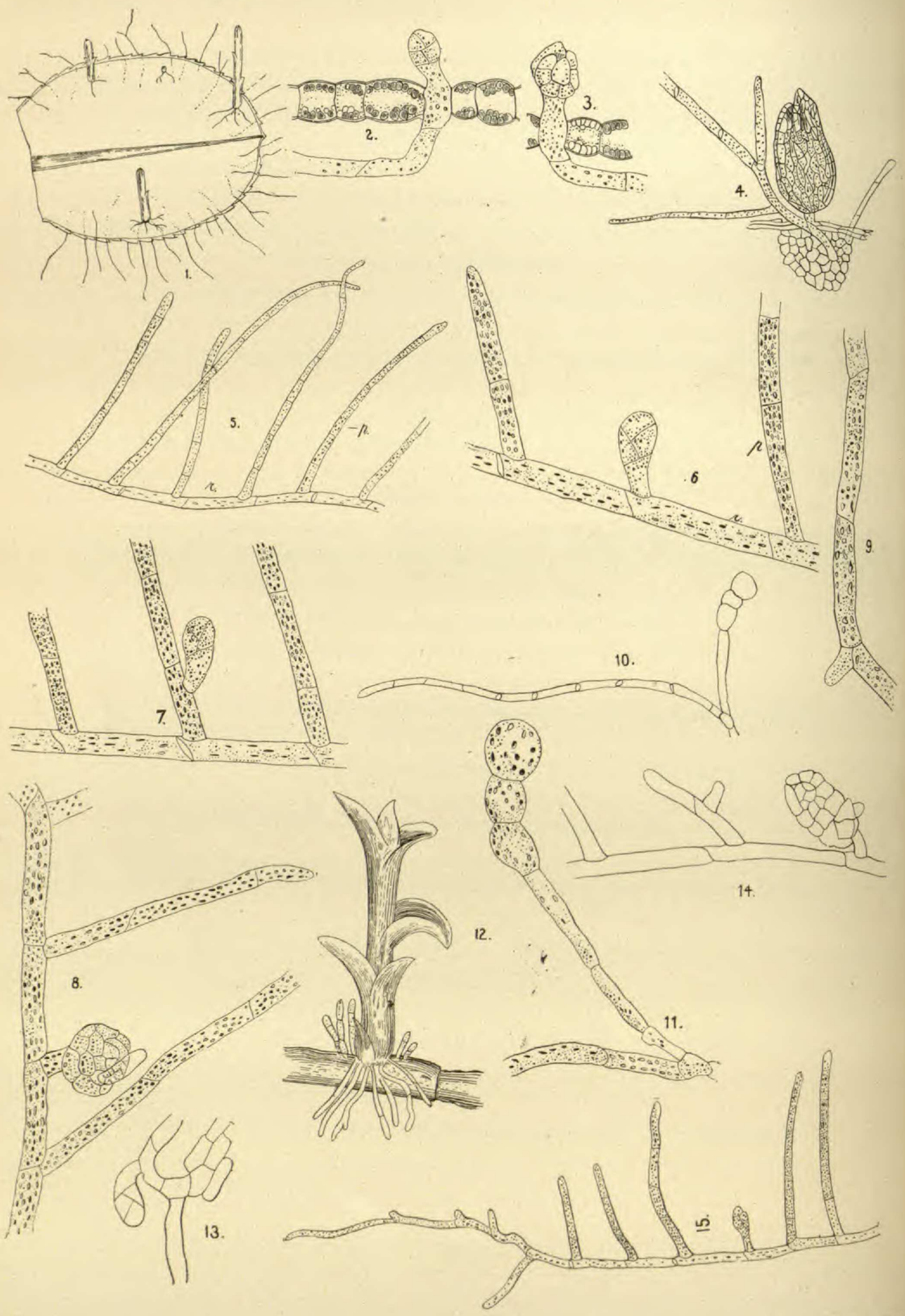
Bryum argenteum.

FIG. 22. Leaf with protonema. × 53.

FIG. 23. A few of the marginal leaf cells showing the origin of protonema. × 130.

FIG. 24. Leaf with protonemata. × 53.

FIG. 25. A few of the basal cells showing the origin of the protonema. × 130.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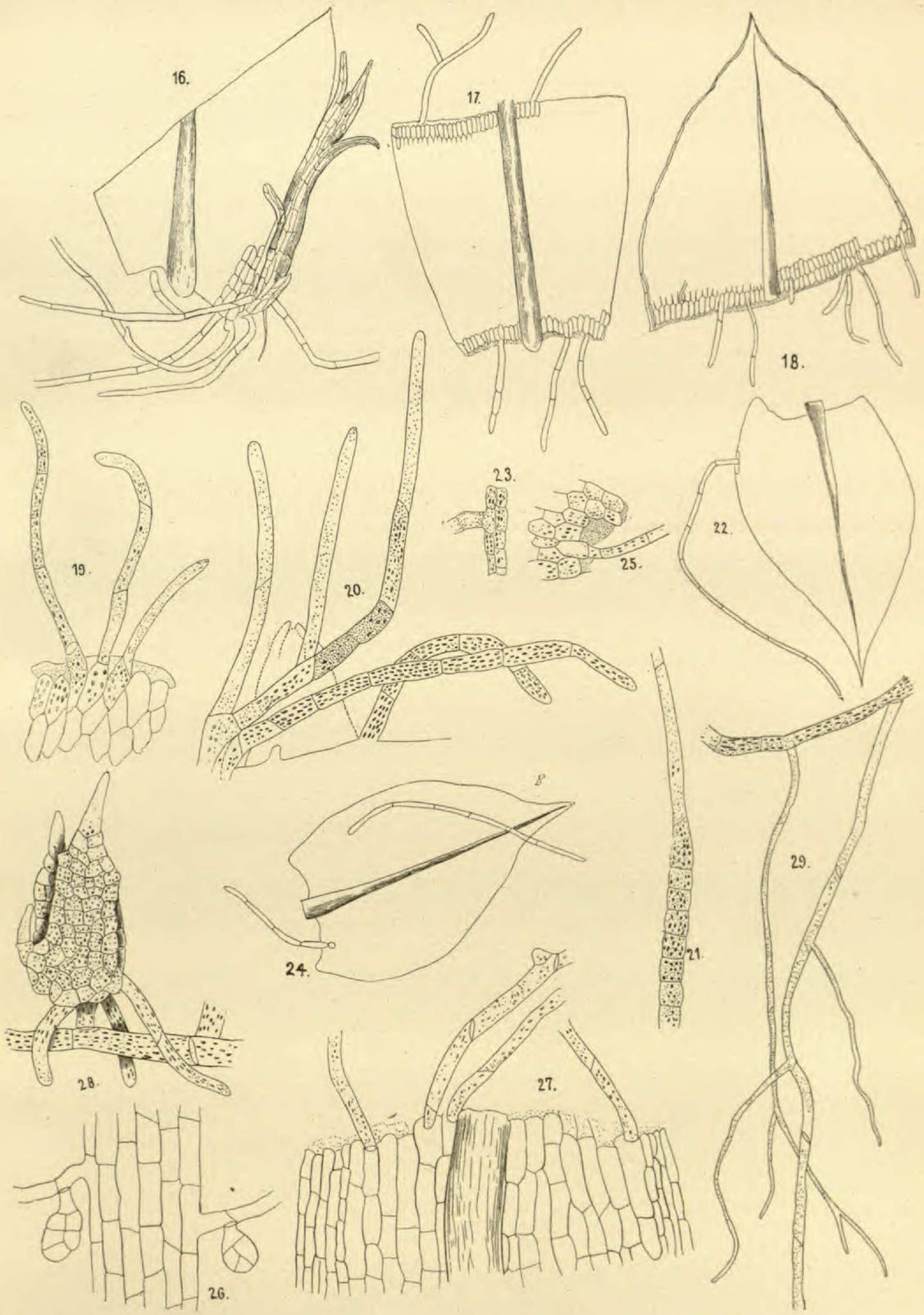
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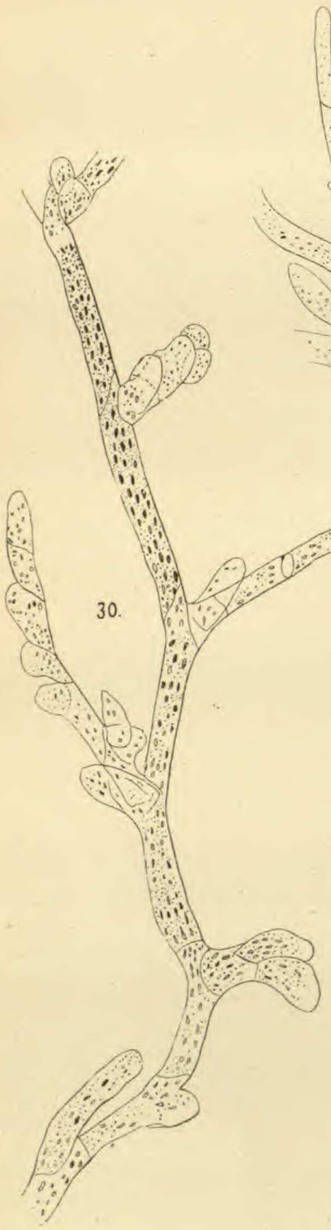
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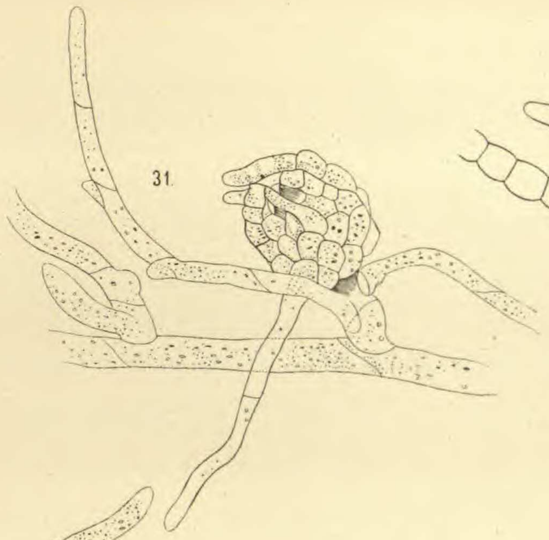
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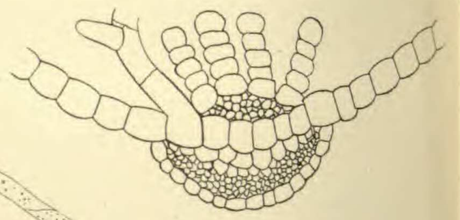
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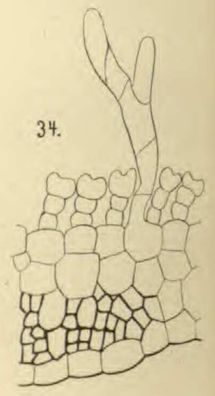
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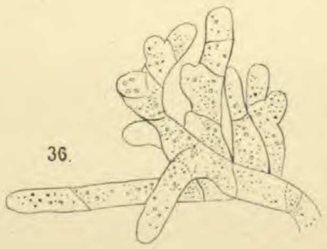
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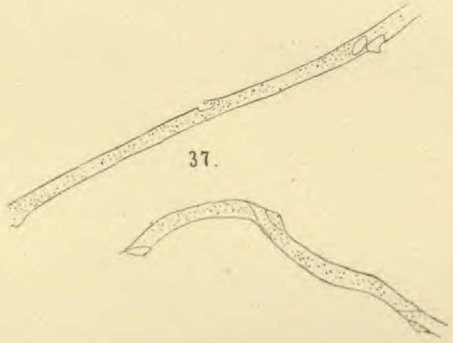
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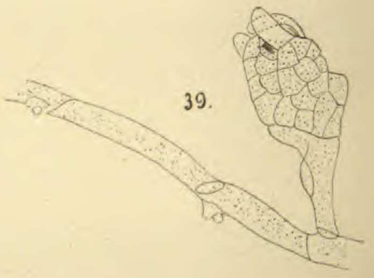
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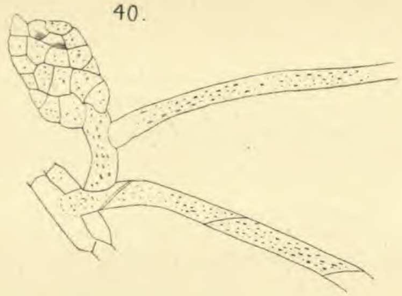
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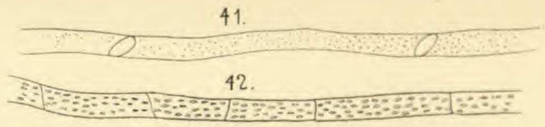
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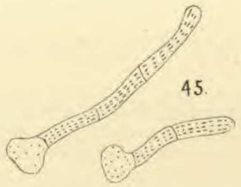
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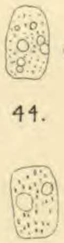
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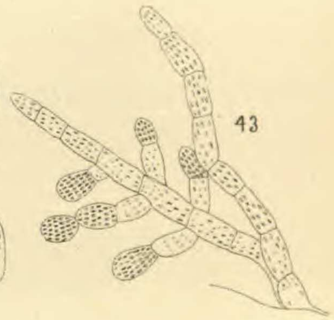
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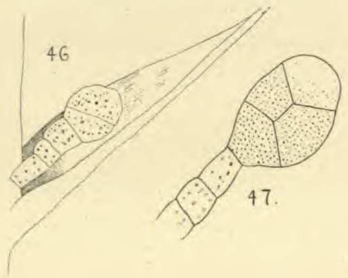
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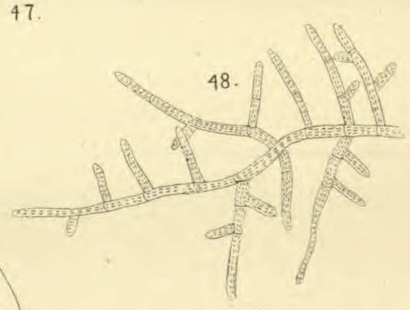
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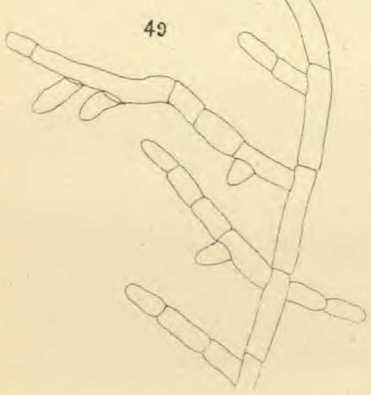
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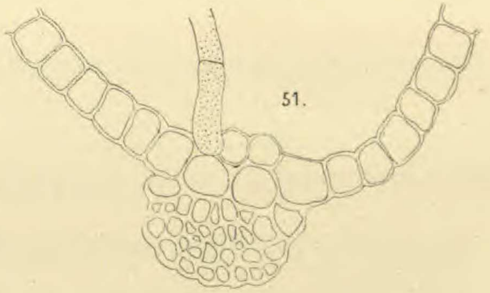
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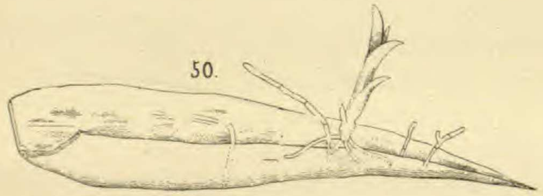
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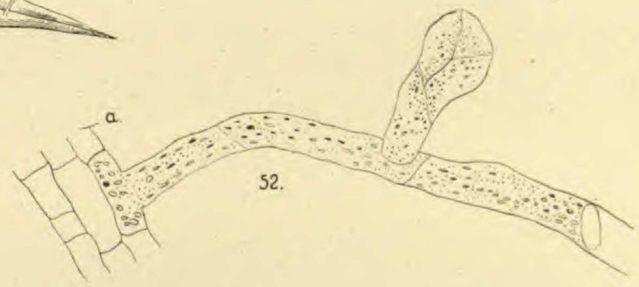
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